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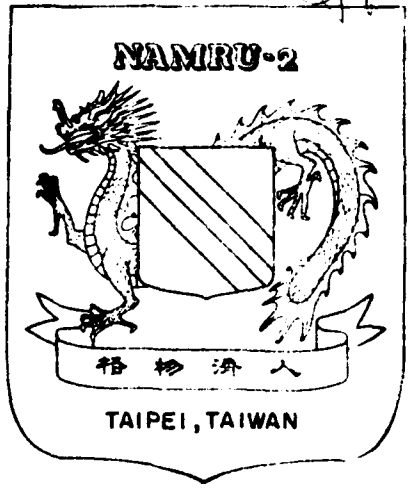

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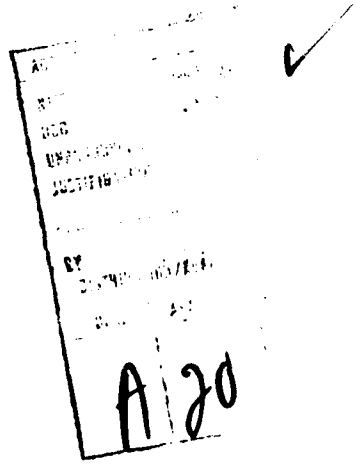

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Running title: Influenza Surveillance in Manila.

ABSTRACT. An outbreak of influenza occurred in Manila, Republic of the Philippines during June through August of 1975. The outbreak was documented by isolation of virus from throat swabs of 14 patients, and by serological testing of unpaired sera. The A/Victoria/3/75 strain of influenza virus was responsible for the outbreak which caused relatively mild symptoms and limited morbidity.

INTRODUCTION

New strains of epidemic influenza A often originate in Southeast Asia during the early summer months, radiate to other areas, and occasionally become pandemic during the winter[1-3]. For this reason the Naval Medical Research Unit No. 2 (NAMRU-2), Taipei, Taiwan maintains an active surveillance program for influenza in this region.

During June, July and August of 1975, influenza virus was isolated from patients in San Lazaro Hospital, Manila, Republic of the Philippines. The isolates were characterized antigenically similar to A/Port Chalmers/1/73 (H_3N_2), the strain which was prevalent during 1974 and 1975[4]. It is now clear that the Philippine strains were antigenically distinct from A/Port Chalmers/1/73 and are more closely related to A/Victoria/3/75 (H_3N_2).

MATERIALS AND METHODS

Throat swabs and sera were obtained from patients at San Lazaro Hospital. Specimens were frozen and stored at —20°C until they could be transported on dry ice to NAMRU-2, Taipei.

Patients were those who, according to the clinical staff, exhibited influenza syndrome. Throat swabs were placed in brain-heart infusion (BHI) broth containing 100 units/ml penicillin, 100 ug/ml streptomycin, and 25 units/ml mycostatin. Virus isolations were attempted in 10-11 day old embryonated chicken eggs following standard procedures[5]. Identification of hemagglutinating agents was accomplished by the hemagglutination-inhibition (HI) microtiter method[5]. Prototype strains were obtained from the World Health Organization (WHO) Collaborating Center for the Americas, Center for Disease Control, Atlanta, Georgia. Antisera were prepared in roosters, treated with 20% kaolin, and absorbed with a 50% suspension of guinea pig red blood cells (GPRBC) to remove non-specific inhibitors. A 0.5% suspension of GPRBC was used to detect agglutination. Selected strains were sent to the WHO Collaborating Center for the Americas for confirmation and identification.

Venous blood specimens were drawn at the same time throat swabs were taken and the serum separated by centrifugation and pipetted into screw-topped vials. Because of the short duration of clinical illness experienced among patients with influenza syndrome, convalescent blood specimens taken 14 days or more after onset were not obtained. In-

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stead, we used a method for rapid identification of influenza outbreaks which requires only single sera[5]. Single sera collected during June and July were tested by the HI test using the A/Hong Kong/5/68 (H_3N_2) strain of influenza virus. Serological results were analyzed by time after onset of symptoms. Specimens obtained 4 days after onset were categorized as "acute" and those obtained 5 or more days after onset were considered "convalescent". Patients in the 2 groups were age matched and geometric mean titers (GMT) were calculated and compared using Student's T-test for the difference of 2 means.

RESULTS

Virus Isolation. Thirteen strains of influenza virus were isolated during 1975. Monthly isolation rates presented in Table 1 show that influenza activity began in June and continued through August.

Virus Identification. 1975 Philippine strains 1-5 were characterized by HI and found to be more like A/Port Chalmers/1/73 (H_3N_2) than any other strain tested (Table 2). Subsequently, these strains were identified by the WHO Collaborating Center for Americas as significantly different from A/PC/73 and more closely related to isolates from New Guinea, Australia, and Taiwan (Table 3). Philippine strains 6-12 were tested by HI including rooster antisera prepared against A/Philippines/1/75 and found to be closely related (Table 2).

Serology. The GMT of acute specimens collected during the month of June was 3.11 while the GMT of convalescents was 3.38. There was no statistically significant difference between these means $0.4 < p > 0.3$. This was surprising since an outbreak had been documented by recovering virus isolates from clinical material during this period. The procedure was repeated using specimens obtained after the onset date of the first etiologically proven case. The GMTs were 2.84 and 3.38 for acute and convalescent groups, respectively. The difference between these means was statistically significant at the $p < 0.2$ level. The GMT of acute and convalescent sera obtained from patients who had onset in July were 2.92 and 3.46. This difference was highly significant ($p = 0.05$). There was not a sufficient number of sera available from August to employ the test.

DISCUSSION

An outbreak of influenza occurred in Manila, the Republic of the Philippines following one in Taipei, Taiwan Province, Republic of China[6]. Both epidemics lasted less than 2 months and were not associated with

large scale morbidity or with unusually serious symptoms. Each outbreak was caused by strains of influenza virus similar to A/Victoria 3/75, the strain responsible for widespread outbreaks during 1975 and 1976[7]. The Manila outbreak was documented by isolation of virus from clinical material and by serological means described here.

The use of unpaired sera of rapid diagnosis of influenza outbreaks was proposed by Grist and co-workers in 1961[8]. They compared antibody titers of patients who had been ill for a week or less with titers of patients who had been ill more than 1 week. A 4-fold difference in titers between those convalescing patients and those acutely ill was considered evidence of an outbreak. A refinement of this technique[5] led to increased sensitivity and employed a statistical test for evaluation of the difference between GMTs of the groups.

The test depends upon the correlation of antibody levels with time after onset of symptoms. Sera obtained from patients with recent onsets should have lower antibody titers than sera from those convalescing from disease. Thus, sera drawn on a single day could be compared and categorized as acute or convalescent depending on time after onset.

In the present study sufficient numbers of sera could not be obtained on the same day, so the period of sera collection was extended. Our results demonstrated that the period of serum collection could be extended to a month without affecting the outcome of analyses. However, because the outbreak began in the middle of a month, the GMTs were not significantly different. Convalescent sera with low antibody titers were drawn before the outbreak began, and inclusion of such sera reduced the difference between acute and convalescent groups. When serological results from only the portion of the month during which influenza cases were virologically confirmed were analyzed, a highly significant difference was found. For purposes of surveillance, a week probably provides enough time to accumulate a sufficient number of specimens and yet provides a short time to detect differences between titers.

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Table 1

Isolation of Influenza Viruses from Throat Swabs

Months of The Year	No. of Specimens Tested*	No of Strains Isolated	% Isolation
January	19	0	0
February	15	0	0
March	9	0	0
April	17	0	0
May	39	0	0
June	39	7	13
July	30	7	23
August	12	1	8
September	5	0	0
October	15	0	0

* Throat swabs were inoculated into 10-day old embryonated chicken eggs.

Table 2

Strain Characterization by Hemagglutination-inhibition

Antigens	Rooster Sera					
	A/HK/68	A/TW/72	A/PC/73	A/Scot/74	A/Phil/75	B/HK/72
A/Philippines/1/75	40	320	640	40	*	10
A/Phil/2/75	< 10	80	160	20	*	< 10
A/Phil/3/75	40	160	320	40	*	20
A/Phil/4/75	< 10	320	1280	10	*	10
A/Phil/5/75	< 10	80	160	10	*	< 10
A/Phil/6/75	20	160	640	10	640	< 10
A/Phil/7/75	20	80	320	40	320	10
A/Phil/8/75	40	160	640	< 10	1280	< 10
A/Phil/9/75	10	80	320	40	320	< 10
A/Phil/10/75	80	320	1280	40	1280	40
A/Phil/11/75	40	160	320	40	640	< 10
A/Phil/12/75	40	320	1280	40	1280	< 10
Homologous antigen and antiserum	160	640	320	40	320	320

* Not tested.

Table 3

Identification of Philippine Strains of Influenza Virus by Hemagglutination Inhibition Tests with Ferret Serum*

Antigens	Ferret Serum				
	A/Phil/3/75	A/TW/2/75	A/Vic/3/75	A/PC/1/73	A/Scot/840/74
A/Philippines/1/75	320	640	640	160	40
A/Phil/2/75	640	640	640	160	40
A/Phil/3/75	320	320	320	80	20
A/Phil/4/75	640	640	640	160	40
A/Phil/5/75	640	640	1280	320	80
A/Taiwan/2/75	640	640	640	160	40
A/New Guinea/1/75	640	1280	1280	160	40
A/Victoria/3/75	320	640	640	160	40
A/Port Chalmers/1/73	40	160	160	1280	80
A/Scotland/840/74	40	20	80	320	640

* Personal communication, WHO collaborating center for Americas, 1976.

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An outbreak of influenza occurred in Manila, Republic of the Philippines during June through August of 1975. The outbreak was documented by isolation of virus from throat swabs of 14 patients, and by serological testing of unpaired sera. The A/Victoria/3/75 strain of influenza virus was responsible for the outbreak which caused relatively mild symptoms and limited morbidity.

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